ELECTRON BEAM PROCESSING ON RAW GRAINS USED IN WHEAT BEER PRODUCTION

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

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One of the key obstacles encountered in the beer brewing industry is the microbial contamination of raw grains used in the production of beer. The barley and wheat grains used in the brewing industry are vulnerable to both bacterial and fungal contaminants. These contaminants are capable of producing defects and inconsistencies in both the quality and safety of the finished beer. Electron beam (eBeam) processing has been proven to lower the natural biological load found in barley grains, however, its effects on wheat grains used for brewing beer are known to lesser extent. Due to the growing popularity of wheat beers amongst consumers, there has been an increase of wheat production for brewing purposes, which may lead to an increase of microbial contamination of wheat crops. eBeam processing may be an efficient technology for reducing microbial contaminants in grains used for wheat beer production. This study will use raw grain samples which will be tested for microbial bioburden (mold and yeasts) and evaluated for "germinative energy" before and after eBeam treatment. The processed grains, along with a control (untreated) group, will then be malted and used to produce multiple batches of a standard wheat beer which will be tested for quality and compared. The hypothesis is that, if the raw grains are eBeam processed at doses between 0 to 12 kGy, there should be a significant reduction of biological contaminants without a significant decrease in the grains malting quality, so as to produce an acceptable wheat beer.

DEDICATION

I dedicate this research to Emma and my parents. Thank you all for your continued love and support. I also want to give special thanks to Chris, my friend and mentor.

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I want to thank Professor Suresh Pillai and Ms. Jessica McKelvey for providing me their expertise and support throughout these studies. Their help has been monumental to me in writing this thesis as well as my development as a researcher.

NOMENCLATURE

APC	Aerobic Plate Count
MYC	Mold and Yeast Count
PCA	Plate Count Agar
GE	Germinative Energy
eBeam	Electron Beam
RH	Relative Humidity
kGy	KiloGray
CFU	Colony Forming Unit
OG	Original Gravity
FG	Final Gravity
ABV	Alcohol By Volume
Wort	Unfermented beer

CHAPTER I

INTRODUCTION

A major concern in the brewing industry today is the incidence of both fungal and bacterial contamination of malted grains used for the production of beer. Contaminants, such as the mold, *Fusarium* spp., can negatively affect the brewing process in a number of ways, both economically, as well as quality of the finished product. Many zero-tolerance policies concerning contaminants in grains have been set by maltsters and brewers alike, resulting in the rejection and waste of a significant amount of grain product every year. Electron beam (eBeam) processing may prove to be an effective and efficient method of lowering the levels of contaminants present in the raw grains before they are malted and used to brew beer.

Grains used in the brewing industry

There are many different assortments of grains that can be utilized to produce beer. The most commonly used grain is barley. However, other grains such as wheat, rice, rye, and sorghum are also good alternatives for brewing beer. No matter which category of grain a brewer chooses to use, the raw grains must first be delivered to a maltster, who converts the grains into malt. The malting process involves the development of enzymes and the conversion of starches into sugars that can be later fermented into carbon dioxide and ethanol. This procedure begins by steeping the raw grains in water to begin the germination process. The germination is halted after a certain period of time and the grains are then moved to a kiln where they are dried and roasted at various temperatures and times, resulting in malted grain. The more heavily roasted grains produce darker malts, which are used to brew darker varieties of beer.

Grain pathogens

A wide array of pathogens exists for grains used in the brewing industry. Contaminants can include anything from bacteria, to wild yeasts and molds. Many of these grain contaminants cannot withstand the high temperatures involved in the malting and brewing procedures. However, there is still cause for concern, as some unfavorable species can still contaminate the post-processed ingredients, or remain in the grains during every step in the production of beer. One example of a particularly problematic contaminant is *Fusarium* spp. *Fusarium* spp. are molds known to be the primary cause of Head Blight in grain crops. They also produce a number of mycotoxins, such as Deoxynivalenol (DON), which is suspected to be a principal cause for "beer gushing", or an uncontrollable cascade of foam and liquid from the container upon opening (Gyllang et al. 1981). DON has also been known to induce vomiting in humans and livestock, which prompts maltsters and brewers to discard any grains that contain DON above the standard limit, 0.5 ppm (Wolf-Hall, 2007). This results in mass quantities of grain being wasted after every new harvest, which equates to significant financial losses to the industry as a whole.

Electron Beam technology

Electron Beam (eBeam) processing is a technology of sterilizing food products by exposing them to a focused beam of electrons. The highly charged electron beam is generated from commercial electricity and accelerating them by means of a linear accelerator. It is a form of ionizing radiation that inactivates microorganisms. The eBeam dose, typically measured in Gray or kiloGray units, can be adjusted so that the microbial levels can be controlled all the way from just disinfecting to pasteurization and even sterilization. eBeam irradiation causes extensive

double strand breaks in the microbial DNA thereby preventing them from multiplying or reproducing any further (Pillai and Shayanfar, 2015). This method of food safety enhancement has been proven to be very effective. This technology is approved by the FDA, USDA, USDA-FSIS, USDA-APHIS and it used around the world. eBeam is widely used by the medical device sterilization industry. NASA also utilizes this technology to sterilize the food that is delivered to the astronauts onboard on the International Space Station.

Previous applications of ionizing radiation on grain products

Previous methods of decreasing pests and contaminants in raw wheat include methods such as irradiation using ^{60}Co and microwave irradiation (Warchalewski et al. 2000). Other researchers have also studied the effect of gamma radiation on the malting quality of barley samples (Koksel et a. 1998). Recent research was performed to reduce biological contaminants like *Fusarium* spp. in standard ales by utilizing eBeam pasteurization on raw barley (Kottapolli et al. 2006). However, as of yet, there has been little knowledge pertaining to the reduction of contaminants and toxins present in grains used to produce standard wheat beers. eBeam technology may serve as a practical method for reducing contaminants in raw wheat and barley used for wheat beer production.

CHAPTER II

METHODS

Processing raw wheat and barley using eBeam

Grain samples

Preliminary trials were performed using samples of raw hard winter wheat, and raw hulled barley, meaning the outer hull of the barley was removed during processing. The samples used for preliminary trials were purchased from an online distributor (Jaffe Bros. Natural Foods, Valley Center, CA). A need for a change in barley varieties led to subsequent barley samples being purchased from a different online distributor (Wheatgraskits.com, Salt Lake City, UT). The grain was separated into two sets of duplicate samples. The first set was used to determine the grains' microbiological load, and included 25 g samples consisting of equal parts barley and wheat. The second sample set was used to test the grains ability to germinate and included groups consisting of 50 individual wheat kernels along with 50 individual barley kernels.

eBeam processing

Electron beam processing of the raw grains was performed at the National Electron Beam Center at Texas A&M University. All samples were placed in plastic Whirl-Pak bags (Nasco, New York, NY) and heat sealed prior to being processed. Irradiation doses were measured using alanine dosimetry (Praveen et al. 2013). Dosimeters (Harwell Dosimeters, Oxfordshire, UK) were placed underneath the sample bags, oriented in the center, but uncovered by any grain. After being exposed to the eBeam, the dosimeters were measured using the Bruker E-scan

spectrometer (Bruker, Billerica, MA) to verify that the samples received the target doses. In order to determine a reasonable dose range for the experiment, the initial test samples were processed using eBeam irradiation at 0, 6, and 12 kGy.

Aerobic plate count testing of processed grains

Aerobic plate counts (APC) assays were adapted from the *FDA's Bacterial Analytical Manual* (Maturin and Peeler, 2001). Using a stomacher, homogenates were made from the 25 g samples by adding 225 ml of 0.1% Buffered Peptone Solution (BPS) to each sample inside stomacher bags, which created a 10^{-1} dilution. The homogenates were serial diluted to 10^{-2}, and 10^{-3} in 0.1% BPS. Then, 0.1ml samples of the dilutions were plated on Plate Count Agar (PCA) plates which were allowed to incubate for 48 hours at 35^{0}C. After incubation, the colonies that had formed were enumerated and the CFU/g was calculated.

Mold and yeast count testing of processed grains

Mold and yeast count (MYC) assays were adapted from the *FDA's Bacterial Analytical Manual* (Tournas et al. 2001). Serial dilutions were prepared in the same manner as previously mentioned in the APC assays. After stomaching, 0.1ml of the dilutions were plated on malt agar plates (Hardy Diagnostics, Santa Maria, CA) and allowed to incubate for 5 days at 25°C in the dark. After incubation, the number of yeasts and molds developing on the plates were enumerated and the CFU/g was calculated.

Germinative energy testing of processed grains

Methods for testing the processed grains' Germinative Energy (GE) were adapted from previous studies (Kottapalli et al. 2006). The processed samples containing 100 kernels of grain (50 of barley, 50 of wheat), and the control group, were placed inside of sterile, glass petri dishes, each containing two sheets of dry filter paper. Next, 4 mL of distilled water was added to each dish to allow the kernels to begin germinating. The dishes were then placed inside a 100% Relative Humidity environment at room temperature for 3 days. The samples were checked for sprouting kernels after 24, 48, and 72 hours. The number of kernels that germinated at the end of 3 days was recorded. The GE was calculated as the percentage (%) of kernels that germinated.

Malting processed grain/brewing beer

Duplicate samples consisting of 1 pound each of both wheat and barley were eBeam processed at the target doses of 0 and 8 kGy. The processed samples then underwent a three-step malting process (Mallet, 2014) (Fig.9 A-D in Appendix). The resulting malt was then used to brew four samples of wheat beer (Fig. 10 A-D in Appendix).

Malting process

In order for the raw grains to become malt, they first underwent a steeping process, which involved alternating, two hour cycles of steeping in water and eight hour cycles of air-drying, for three complete cycles. The steeping increased the seeds' moisture content by approximately 30% and the majority of the grains began to "chit", or sprout small rootlets. Next, the grains were spread out on baking sheets to increase their surface area exposure and kept covered in a cool, moist, well-ventilated environment for 3-5 days as germination took place. Once the germinating

seeds began developing acrospires that were 80-100% the length of the entire kernel, they were then moved to the drying stage. The germinating seeds were dried for six hours using a commercial food dehydrator until the moisture content was at approximately 10-12%. The rootlets of the seeds were removed by sifting with a colander, resulting in finished wheat and barley malt. This malting process activates necessary enzymes within the seeds and reveals carbohydrates within the seeds endosperm, which will eventually be broken down into simple sugars that the yeast then consume to produce CO_2 and ethanol.

Brewing process

Once each sample was malted, the resulting malts were ground into grist using a grain mill (Austin Homebrew Supply, Austin, TX). The grist samples were then used to brew four one-gallon batches of standard wheat beer wort. The wort samples were tested for specific gravity and turbidity. The samples then received equal amounts, approximately ($50 \text{ X } 10^9$ cells) 50 billion cells, of the yeast strain, NorCal Ale #1 Yeast (GigaYeast, Inc., San Francisco, CA). The wort samples were then allowed to ferment in one gallon jars for three weeks.

Finished beer analysis

Once the fermentation was complete, the finished beer was analyzed for specific gravity, turbidity, and pH.

Specific gravity and alcohol by volume

Specific gravity refers to the density ratio of wort/beer to the density of water (Graham et al. 1853). Dissolved sugars will make a solution denser than water. Once those sugars have been

fermented and converted to CO_2 and ethanol, the density of the solution is expected to decrease and be closer to that of water. Original Gravity (OG) readings, the density of wort before fermentation, along with Final Gravity (FG) readings, density of beer after fermentation, are used to calculate the final Alcohol By Volume (ABV) of the beer. Specific gravity readings were measured using a calibrated hydrometer and ABV was calculated by entering OG and FG readings into an online ABV calculator (Brewer's c2007-2016).

Turbidity

The turbidity of each sample was determined by measuring light absorbance, or Optical Density at 600 nanometers, a measurement otherwise known as the OD_{600nm}. Each sample was measured for OD_{600nm} before and after fermentation using a Spectrophotometer (Eppendorf North America, Hauppauge, NY). The results were recorded and compared.

pH

During fermentation, the pH of beer will decrease due to the consumption of buffering materials and the release of organic acids by the yeast (Coote et al. 1975). The pH values of finished ale beer is typically in the range of 3.8- 4.2 (MacWilliam 1975). Each sample of beer, after fermentation, was measured using a standard digital pH meter. Results were recorded and compared.

CHAPTER III

RESULTS and DISCUSSION

Preliminary trials

Initial testing was performed on raw hard winter wheat and raw hulled barley. In order to establish an appropriate dose range for use in subsequent testing, the grain samples were processed with eBeam at target doses of 0 kGy, 6 kGy, and 12 kGy, and then later tested for microbiological load (APC, MYC) and Germinative Energy (GE).

APC and MYC

Fig. 1 summarizes the effect of eBeam on the grains' microbiological load. A 2.92 log reduction of aerobic bacteria was observed at 6.121 and 12.684 kGy. Less reduction was observed in mold and yeast counts; at 6.121 kGy there was a 1.14 log log reduction and at 12.684 kGy there was a 1.32 log reduction. The reason for less reduction in mold and yeast counts may be due to the growth of radio-resistant spores of mold and yeast contained in the raw grains (Shae 2000).

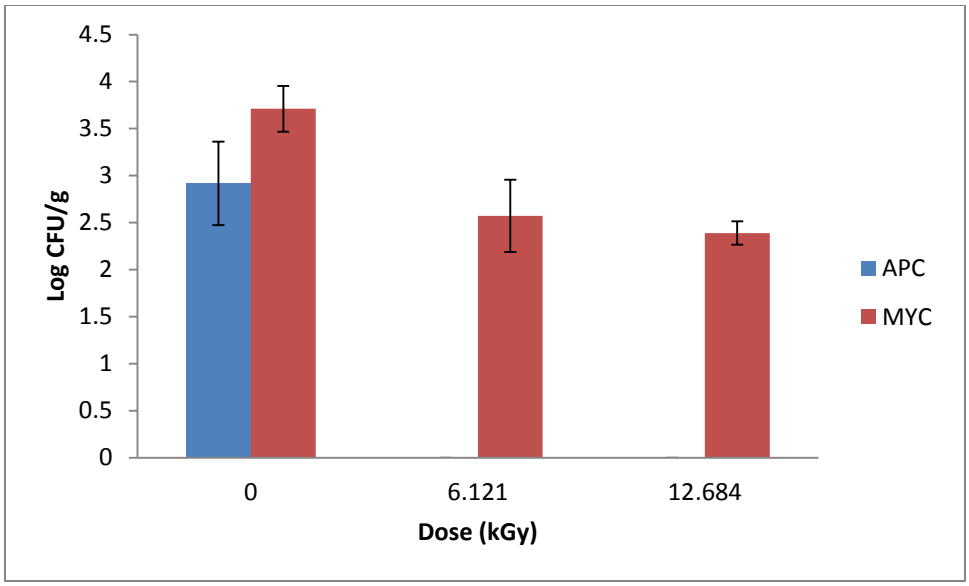

Fig. 1. The effect of eBeam on aerobic plate counts (APC) and mold and yeast counts (MYC) in samples of irradiated wheat and barley. The y-axis values represent the measured doses that each sample received. The Error Bars represent the standard deviation of the average values.

Germinative energy

A major step in the grain malting process is germination. Therefore, it was necessary to test the eBeam processed grains for changes in Germinative Energy (GE). Fig. 3 summarizes the effect of eBeam processing on the Germinative Energy of wheat and barley, respectively. eBeam processing at 5.864 kGy did not affect wheat, but it caused a 14% decrease in the barley's ability to germinate. At 12.281 kGy, there was a 72% decrease in GE for barley and a 47% decrease in GE for wheat. eBeam processing appeared to affect the germination of barley more so than the wheat. This may be due to the fact that the barley was hulled, resulting in increased eBeam dose received by the barley's endosperm.

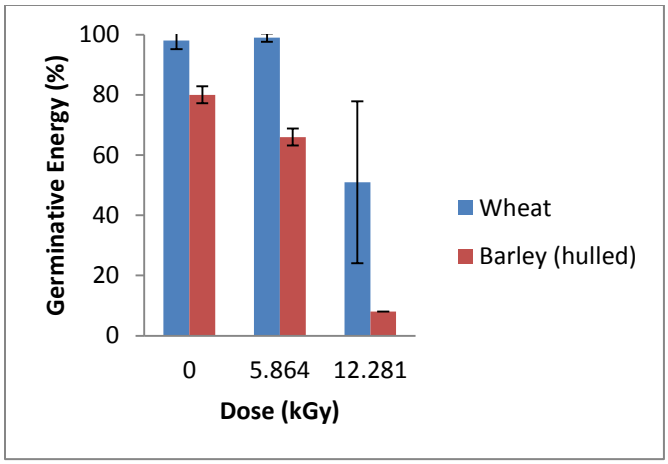

Fig. 3. The effect of eBeam on the Germinative Energy (GE) of both wheat and barley samples. The blue bars represent the average percentage of wheat grains that were able to germinate after 72 hours, and the red bars represent the average percentage of barley grains that were able to germinate after 72 hours. The y-axis represents the actual measured doses that each sample received. The Error Bars indicate the standard deviation of each duplicate sample tested in two trials.

Optimized eBeam processing

After compiling the data gathered during the preliminary trials, a dose range between 0 kGy and 6 kGy was determined to be the most applicable dose for subsequent trials. This decision was made because no significant microbial reduction was observed above 5.864 kGy, and noticeable decreases were observed in the GE of grains that were eBeam processed at 12.281 kGy. It was also necessary to switch varieties of barley samples from the hulled barley to whole barley, meaning the outer hull still remained on the seeds. This is more applicable since the barley used in beer brewing typically possesses intact hulls. After processing and testing the grains containing the new barley variety, the upper limit of the dose range was raised to 10 kGy to increase microbial reduction while still retaining the grains' ability to germinate.

Aerobic Bacterial Counts

Fig. 3 summarizes the effect of eBeam on aerobic bacterial contaminants found on wheat and barley grains at doses ranging from 0 kGy to 10.382 kGy. At 0 kGy, a much higher microbial load was observed in the barley/wheat homogenates than in previous trials. The cause of this may be due to the fact that the barley variety that was used in the preliminary tests was more processed (hull removed) than the new barley variety (hull remained) being tested. Log reduction (1.71 log) was observed at 1.899 kGy. Log reduction (6.08 log) was observed at 7.794 kGy, but no additional reduction was shown in doses above 8 kGy. Kottapolli et al. 2006, demonstrated values of reduction similar to the ones observed in this experiment, with reductions of up to 2 logs at 2 kGy and 5 logs at 10 kGy. The levels of bacterial reduction that was achieved with eBeam processing can be beneficial in reducing defects in beer caused by post-harvest grain pathogens.

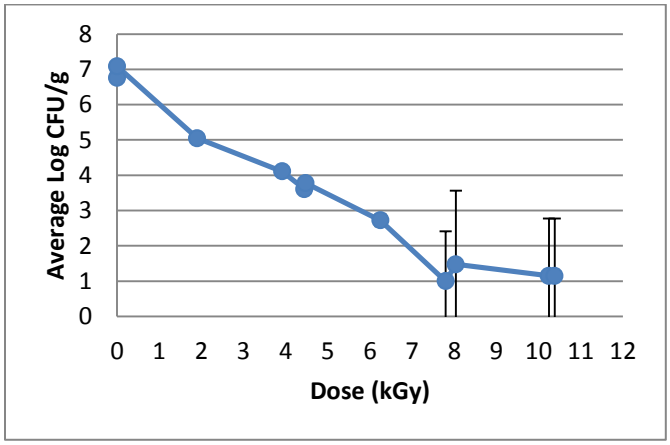

Fig. 3. The effect of eBeam on aerobic plate counts (APC) at doses ranging from 0 kGy to approximately 10.4 kGy. The points on the graph represent the actual measured doses each sample received and the average log CFU/g observed at those respective doses. The Error Bars indicate the standard deviation of the duplicate samples that were tested in three trials. The data represents combined microbial loads of wheat and barley (Stomacher samples).

Effect on mold and yeast counts

Fig. 4 summarizes the effect of eBeam on mold and yeast colonies found on wheat and barley grains at doses ranging from 0 kGy to 10.382 kGy. Significant reduction (1.27 log) was observed at 1.899 kGy, with more reduction (2.64 log) occurring at 10.382 kGy. According to the data, reduction in the mold and yeast was lower than in the aerobic bacterial reduction. This can most likely be attributed to the increased tolerance to irradiation that mold and yeast spores possess, which has been observed in previous studies (Monk et al. 1995) where reduction of mold and yeast remained unchanged after 5 kGy. The reduction of mold and yeast that eBeam processing has displayed can lower levels of pathogenic molds like *Fusarium* spp. and the mycotoxin DON.

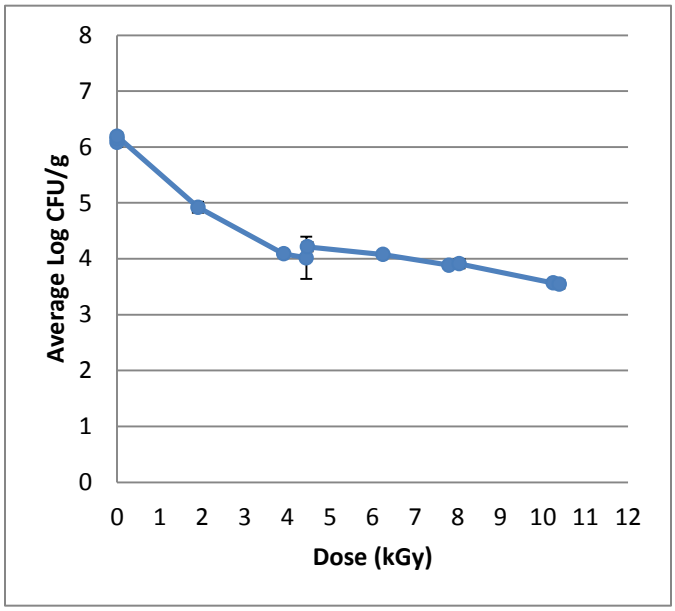

Fig. 4. The effect of eBeam on mold and yeast counts (MYC) at doses ranging from 0 kGy to 10.382 kGy. The points on the graph represent the actual doses the grains received and the average log CFU/g that was observed at those respective doses. The Error Bars indicate the standard deviation of the duplicate samples that were tested in three trials.

Germinative energy

Fig. 5 summarizes the Germinative Energy (GE) of both the wheat and barley grains after exposure to eBeam at varying doses between 0 kGy and 10.382 kGy. The barley grains ability to germinate was much less affected by eBeam irradiation than in previous trials, most likely due to the fact that the new barley variety was less processed than the old variety prior to being treated with eBeam. Both wheat and barley showed high amounts (97-100%) of germination at doses between 0 kGy and approximately 8 kGy. A slight decrease in GE of both wheat and barley was observed at approximately 10 kGy, with values reaching as low as 96-97%. These results are similar to those found in previous studies performed on grain germination after eBeam processing (Sitton et al. 1995). Although, the grains were able to germinate at higher doses of eBeam, there was slight decreases in the rate and degree at which the processed grains germinated (Fig. 11 in Appendix), which in turn may affect the malting quality of those grains.

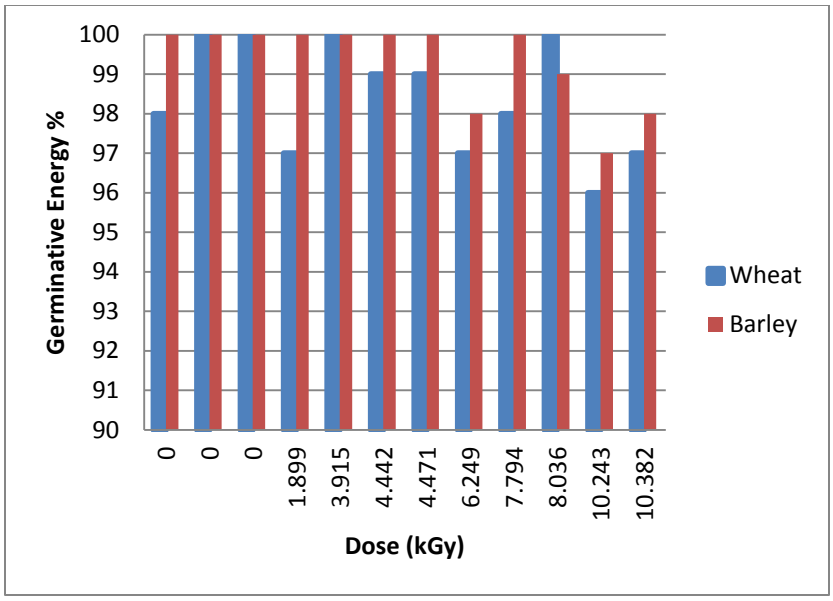

Fig. 5. The effect of eBeam on germinative energy (GE) on both barley and wheat grains. The blue bars represent the GE of wheat while the red bars represent the GE of barley. The x-axis represents the actual dose each sample received. The y-axis represents the GE percentage observed at those respective doses.

Wort/beer assays

Specific Gravity

Specific gravity readings show major differences in the amount of dissolved sugars in solution for the wort samples. Fig. 6 summarizes the findings of gravity measurements along with the calculated final ABV. As shown in the chart, the samples of wort made from grains treated with eBeam at 8 kGy were less dense than the control groups, meaning that there were fewer dissolved sugars in solution. After fermentation, the control samples were less dense than the experimental group, meaning a greater number of the dissolved sugars in the control samples were fermented by the yeast. The resulting ABV for the control group was between 4.2-4.33%, while the ABV for the experimental group was much lower, between 0.79- 1.18%. A possible explanation for these results could be that the eBeam treatment reduced the grains' diastatic power, resulting in less starches being broken down into fermentable sugars (Kottapalli et al. 2006). As the chart shows, even with the small amount of sugars in solution in the experimental

group, very little ended up being fermented. This phenomenon may have occurred because not all of the solutes in the experimental wort were fermentable, and remained unaffected by the yeast.

Targeted eBeam Dose	Specific Gravity		Calculated Alcohol By Volume
	Before Fermentation (OG)	After Fermentation (FG)	
0 kGy (A)	1.040	1.007	4.33%
0 kGy (B)	1.040	1.008	4.20%
8 kGy (A)	1.024	1.018	0.79%
8 kGy (B)	1.020	1.011	1.18%

Fig. 6. Specific Gravity readings before (OG) and after (FG) fermentation along with the calculated ABV for each sample.

Turbidity

As Figure 7 shows, the fermentation process lowered the OD_{600nm} values for each sample, meaning each sample was clarified, or made less turbid, by fermentation. However, the measured OD_{600nm} values were much higher in the experimental group than the control group, both before and after fermentation. A possible explanation for this might be that eBeam processing lowered the amount of the enzyme, alpha-amylase, within the grains, which decreased the amount of long starch chains being broken down into simpler forms, resulting in a more turbid solution (Kottapalli et al. 2006). These results suggest that doses of 8 kGy and above are not ideal for grains used in the brewing industry.

OD_{600nm}	OD_{600nm}	
	Before Fermentation	**After Fermentation**
0 kGy (A)	1.6819	0.1825
0 kGy (B)	1.3732	0.1320
8 kGy (A)	2.8943	1.4865
8 kGy (B)	2.1277	1.4863

Fig. 7. The OD_{600nm} values for each sample, before and after fermentation. More turbidity in experimental samples suggest that less starch in the wort was broken down via enzymatic activity, which will ultimately have a negative impact on the final product.

pH

As seen in Fig. 8, the pH of the 8 kGy beer samples was on the higher end of the typical pH scale for ales (pH 3.8- 4.2). Although the pH values of the 8 kGy samples were not atypical from normal ales, they were on average 0.33 higher than the control group pH values. The differences in pH were due to the lower yeast activity in the 8 kGy samples, which results in less organic acids being created during fermentation (Coote et al. 1975).

Targeted eBeam Dose	**pH**
0 kGy (A)	3.88
0 kGy (B)	3.96
8 kGy (A)	4.14
8 kGy (B)	4.36

Fig. 8. The pH readings of fermented beer made from grains treated with 8 kGy as well as beer made from the control group

CHAPTER IV

CONCLUSION

Practically of eBeam use in the brewing industry

The results of this experiment prove that eBeam processing can indeed decrease the overall microbial levels of bacteria, mold, and yeast on raw grains, without making the grains incapable of germinating. However, at doses of 8 kGy or higher, the malting qualities of wheat and barley grains are negatively affected and do not produce a wheat beer that would be considered generally acceptable for commerce by current malting standards and processes. Because of these findings, it can be assumed that using eBeam processing at doses greater than 8 kGy on raw grains used to produce wheat beer is not practical. An adjustment to eBeam dose along with changes in the malting process can lead to a balance of contaminant reduction and malt quality preservation.

Future research needs

For future research on this topic, it is suggested that more grain samples that are eBeam proccessed at lower doses (2 kGy - 6 kGy) should be used for the malting and brewing stages of the experiment. Also, alternate malting methods should be used for the eBeam processed grains in order to achieve higher quality malt for subsequent brewing stages. Because DON is a major mycotoxin of concern in the brewing industry, future trials should involve measuring levels of DON before and after eBeam treatment. In order to gain more insight on eBeam's effects on the biochemical properties of the grains, it is recommended that future trials make use of Gas Chromatography assays on wort and beer samples.

REFERENCES

Brewer's Friend: Alcohol by volume calculator. c2007-2016. San Marcos (TX): Brewer's Friend; [accessed 2016 Mar 27]. http://www.brewersfriend.com/abv-calculator/

Coote, N. Kirsop, B.H. 1976. Factors responsible for the decrease in pH during beer fermentation. J. Inst. Brew. 82: 147-153.

Graham, T. Hofman, A.W., Redwood, T., 1853. Report on original gravities. Q. J. Chem. Soc. Lond. 5, 229-256.

Gyllang, H. Kjellen, K., 1981. Evaluation of fungal contaminations on barley and malt. J. Inst. Brew. 87, 248-251.

Koksel, H., Celik, S., 1998. Effects of gamma irradiation of barley and malt on malting quality. J. Inst. Brew. 104, 89-92.

Kottapalli, B., Wolf-Hall, C.E., Schwarz, P., 2006. Effect of electron-beam irradiation on the safety and quality of Fusarium-infected malting barley. Int. J. Food Microbiol. 110, 224–231.

MacWilliam, I.C., 1975. pH in malting and brewing. J. Inst. Brew. 81: 65-70

Mallet, J. 2014. Malt: a practical guide from field to brewhouse. Boulder (CO). Brewer's Publications.

Maturin, L., & Peeler, J. T., 2001. Chapter 3, aerobic plate count. BAM (Bacteriological Analytical Manual). US Food and Drug Administration, Washington, DC.

Monk, J. D., L. R. Beuchat, and M. P. Doyle. 1995. Irradiation inactivation of food-borne microorganisms. J. Food Prot. 58,197-208.

Pillai, S.D., and S. Shayanfar, 2015. Electron beam pasteurization and complementary food processing technologies. (eds). Woodhead Publishing, Cambridge, UK. Pp 324.

Praveen, C., Dancho, B., Kingsley, D., Calci, K., Meade, G., Mena, K., Pillai, S., 2013. Susceptibility of murine norovirus and hepatitis a virus to electron beam irradiation in oysters and quantifying the reduction in potential infection risks. Appl. Environ. Microbiol. 79, 3796-3801.

Shea K.M. 2000. Technical report: irradiation of food. Pediatrics 106:1505–10.

Sitton, J. W., Borsa, J., Schultz, T. R., and Maguire, J. D. 1995. Electron beam irradiation effects on wheat quality,seed vigor, and viability and pathogenicity of teliospores of Tilletia controversa and T. tritici. Plant Dis. 79, 586-589.

Tournas, V., Stack, M., Mislivec, P., Koch, H., Bandler, R., 2001. Chapter 18, yeasts, molds and mycotoxins. BAM (Bacteriological Analytical Manual). US Food and Drug Administration, Washington, DC.

Warchalewski, J., Pradzynska, A., Gralik,J., Nowrot, J., 2000. The effect of gamma and microwave irradiation of wheat grain on development parameters of some stored grain pests. Nahrung 44. 6: 411-414.

Wolf-Hall, C., 2007. Mold and mycotoxin problems encountered during malting and brewing. Int. J. Food Microbiol. 119, 89-94.

APPENDIX A

Fig.9 A) The grains underwent a steeping process in order to increase their overall moisture content and begin the process of germination. B) Grains were spread out on a baking sheet in order to increase their surface area exposure, which aids in the respiration of germinating seeds. C) Grains were kept in trashcan liners to maintain a humid environment during germination. Grains were stirred twice a day to prevent mold growth. Grains germinated for 3-5 days, until the majority of seeds developed acrospires that were 80-100% the length of the kernels. D) The germinating seeds were dried using a commercial food dehydrator set at 110°F (43.3°C)

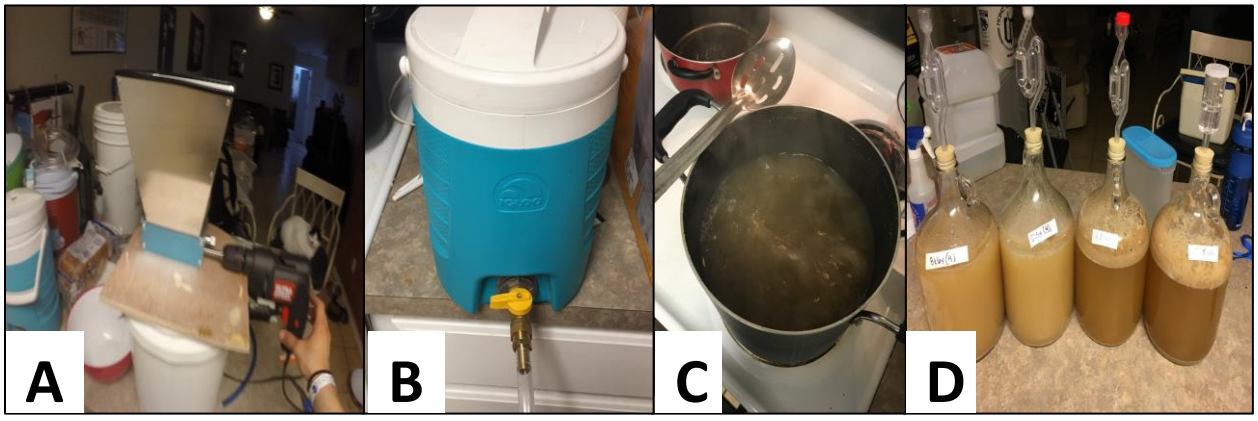

Fig.10 A) The malts were ground into a grist using a cereal grain mill. The milling breaks up the dried malt, exposing the starches and enzymes found within the hulls. B) The Mash Process. The grist was transferred to a 2 gallon mash tun, where it was mixed with hot water and kept at a constant temperature, 153°F (67°C), for 1 hour. This process allows diastatic enzymes to break down starches into simple sugars, which are then dissolved into solution, resulting in wort, or unfermented beer. C) The Boil Process. The wort is then boiled for 1 hour. The wort is condensed and sterilized. This is also the stage where hops are added. Acids extracted from the hops during the boil contribute bittering, aromatic, and preservative properties to the finished beer. D) The wort was cooled to room temperature before equal amounts of yeast were added to each sample. Air locks were placed on each jug to allow CO_2 to vent outward, while keeping the samples protected from the outside environment. All samples were allowed to ferment for 3 weeks. The two samples on the left were made from the grains that received eBeam doses of 8 kGy. The two samples on the right were made from the control group.

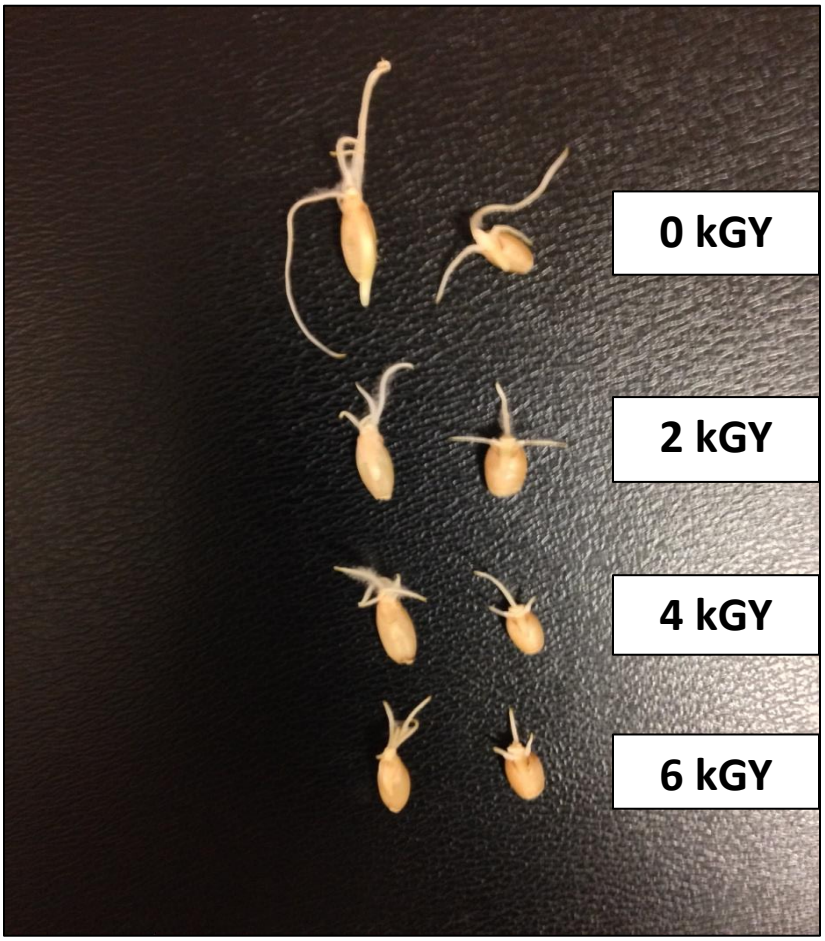

Fig. 11. Differences in germination were observed in grains that had been eBeam processed. Decreased growth and decreased rate of growth were evident with increased dosage of eBeam.